Expression, Purification, Refolding, and Structural Investigation of the Membrane Protein, Lipoprotein Signal Peptidase (LspA) by Solid State NMR

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Introduction

Lipoprotein Signal Peptidase (LspA) is a 21kDa membrane protein located in the cell inner membrane. It contains four predicted transmembrane helices and the potential catalytic residues at the extracytoplasmic membrane surface specifically catalyze removal of the signal peptide from glyceride-modified prolipoproteins.

Experimental

Here is a procedure for making LspA samples for the structural studies by solid state NMR. LspA is expressed *in E.Col*, purified and refolded with detergent on a Ni-NTA affinity column and uniformly aligned in lipid bilayer on glass slides. Both gNhsqc experiments for monitoring refolding of LspA and 1D spectra of the uniformly aligned LspA samples in lipid bilayer were run respectively on the 720 MHz, solution NMR spectrometer, and on the 600MHz solid state NMR, respectively at the National High Magnetic Field Laboratory, Tallahassee.

Results and Discussion

Final concentration of reconstituted LspA of up to 35mg/ml has been achieved. Reconstitution of LspA in detergent micelles was monitored by CD and solution NMR HSQC. The dispersion of its resonances indicates the reconstituted LspA in DPC micelles has primarily alpha helical features. The number of the resonances in the gNhsqc spectrum shows LspA is uniformly labeled by the present expression procedure. For the experiment of aligned LspA in lipid bilayer, the different lipids, pH value, and the ratio of the protein to lipid were screened. Preliminary 1D solid state NMR spectrum of LspA in either 10:1 or 5:1 ratio of LspA to lipid (weight to weight) composed of 4:1 palmitoyloeoylphosphatidylglycero (POPG) and palmitoyloleoylphocholine (POPC) at pH9.1 give high degree of alignment.

Conclusions

Based on the results and discussion above, more experiments for full length LspA alignment in lipids bilayer will be continued and especially 2D solid state NMR PISEMA-HECTOR experiment will be run to show resonance patterns known as PISA wheels for the structural studies of transmembrane helices of LSPA.

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References

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